**A Practical Manual**

On

**“SOIL FERTILITY & NUTRIENT MANAGEMENT”**

NRM-121

**For B.Sc. (Hons.) Horticulture 2nd Semester**





**2022**

DEPARTMENT OF NATURAL RESOURCE MANAGEMENT,

COLLEGE OF HORTICULTURE, BERMIOK, (C.A.U., IMPHAL)

SOUTH SIKKIM-737134

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**Prepared By:**

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*The views expressed in the manual are the personal opinion of the contributors*

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# FOREWORD

**The Earth, our unique home in the vastness of universe is in crisis. We owe our very existence to the thin but precious skin of the Earth’s surface called Soil. It is in this thin layer of soil that the plant and animal kingdoms meet the mineral world and establish a dynamic relationship. Plants obtain water and essential nutrients from the soil and animals depend on plants for their lives. Plant and animal residues find their way back to the soil and are decomposed by the teeming microbial population living there. As such, life is vital to soil, and soil is vital to life.**

**Soil promotes and sustains life in its various forms. Early chemists considered soil as a storehouse for plant nutrients. Soil fertility i.e. plant nutrients available in the soil needs to be understood and studied properly because there is an urgent growing need to produce food for the world’s growing population. Therefore, management of soil fertility is very much crucial to maintain the productivity and profitability of cropping systems.**

 **Anupam Mishra**

**PREFACE**

Soils are crucial to life on earth. To a great degree, the quality of the soil determines the nature of plant ecosystems and the capacity of land to support animal life and society. The human population increases day by day but the resource base is decreasing because of soil degradation and urbanization. In order to survive as a species, we will have to greatly improve the efficiency and sustainability with which we manage our soil resources. In this context, soil fertility and nutrient management is one of the most important practice to sustain the productivity of soil.

The main purpose of this manual is to create an awareness to become familiar with the important parameters of soil fertility. This manual has been prepared for the students of 2nd Semester B.Sc. (Hons.) Horticulture according to the Fifth Dean’s Committee Report. The manual contains the analytical procedures and its principles presented in a simple and lucid way for measuring different parameters of soil fertility.

**Chakpram Birendrajit**

#### SYLLABUS

# Analysis of soil for organic matter, available N, P, K and Micronutrients and interpretations. Gypsum requirement of saline and alkali soils. Lime requirement of acid soils. Estimation of organic carbon content in soil. Determination of Boron and chlorine content in soil. Determination of Calcium, Magnesium and Sulphur in soil. Sampling of organic manure and fertilizer for chemical analysis. Physical properties of organic manure and fertilizers. Total nitrogen in urea and farmyard manure. Estimation of ammonical nitrogen and nitrate nitrogen in ammoniacal fertilizer. Estimation of water soluble P2O5, Ca and S in SSP, Lime and Gypsum. Estimation of Potassium in MOP/SOP and Zinc in zinc sulphate. Visiting of fertilizer testing laboratory.

**CERTIFICATE**

#### This is to certify that Mr./Ms ………………………………….……………........……….

#### Reg. No…………………….……has performed Practical for the semester ………………..

#### B. Sc. (Hons) Horticulture in the Course No………….………………………………………. Title……………………………………………………………..………………………………

####  During the academic year…………………………

#### He/She has performed……………………. practical out of ……………………

#### US ID : ………………………..

####

####  Course Teacher

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**Experiment 1: Safety Measures In The Laboratories**

Working in the laboratory involves the use of chemicals, glassware, other apparatus and sophisticated equipment. To avoid accidents the it is advised to take the following precautions:

1. Use apron or laboratory coat
2. All acids are highly corrosive and alkalies highly caustic. Therefore, use them carefully and do not spill over your clothes, body or on the tables.
3. Never add water to the concentrated acid, always add acid to water make required solution.
4. Never open ammonia solution at room temperature. It should be opened after proper cooling to avoid its explosion.
5. Handle glassware with care. Broken glass may cause injuries on the parts of your body. Handle them properly and avoid its breakage.
6. Many chemicals are highly poisonous. Therefore, do not suck them with mouth. Use suction bulbs.
7. Instruments are very costly and their repair is very time consuming. Therefore, handle them carefully. Get the guidance from your instructor.
8. Perform the exercises at the seats allotted to you Do not form groups. Avoid unnecessary any loud talk.
9. Approach your instructor for any clarification and help.
10. Take help of laboratory attendant for your requirement of chemicals, solutions and

 glassware etc.

11. In spite of these precautions if any mishap occurs during the conduct of the practical, adopt the following safety measures of first aid.

**Eye accident**

In all cases consult the doctor. Meanwhile first aids should be done.

**(a) Acids in the eyes**

Wash the eye with 1% NaHCO3 if the acid is dilute. In case of concentrated acid the eye should first be washed with a large amount of water and then thoroughly with the bicarbonate solution.

**(b) Caustic alkali in the eye**

Proceed as for acid in the eye, but wash with 1% boric acid solution instead of bicarbonate.

**Fires**

Make the victim lie down on the floor and wrap with a blanket firmly around the burning clothes until the fire is extinguished.

**Burning reagents**

Turn out all gas burners and switch off all electric hot plates in the vicinity. Remove the things which may burn. Dry sand may be applied if the extinguisher is not readily available.

**Poisons**

1. **Acids**

If acid is sucked in the mouth, drink a lot water, followed by lime water or milk or magnesia

1. **Intake of caustic alkalis**

Drink a lot of water followed by vinegar, lemon or orange juice, solution or citric or lactic acid.

1. **Other poisons**

if salts of heavy metals have been swallowed, give milk or white of an egg. If compounds of arsenic or mercury have been swallowed, give emetics like one teaspoon full of mustard, or one teaspoon full of common salt or zinc sulphate, in a tumbler of warm water. Rush immediately for medical helpful.

Burns

1. **Cloth burns**

Apply tannic acid jelly (Tannatak), acriflavine (Burnol) or butesin picrate ointment on slight burns caused by dry heat in which the skin is not broken. For major burns acriflavine jelly or crystal violet may be applied.

1. **Skin burns due to acid**

First wash with water thoroughly, then with saturated solution of NaHCO3 and finally with water. if acid burns are serious, apply any disinfectant after the washing treatment. Allow the skin to dry and cover all the burn skin with burnol.

1. **Alkali on the skin**

Same treatments as in the case of acid burns except that in second washing use 1% acetic acid instead of saturated NaHCO3 solution.

**Organic substances on the skin**

Wash thoroughly with rectified spirit followed by soap and warm water.

**Cuts**

Allow minor cuts to bleed for a few seconds and then remove the glass piece if any. Apply a disinfectant and bandage. In serious cuts, consult the doctor at once after washing the part with a disinfectant.

**Experiment 2: Determination of pH in soils**

 Determination of pH is actually a measurement of hydrogen ions activity in soil water system. It is defined as negative logarithms of the hydrogen ions activity.

Mathematically, it is expressed

 pH = - log a H+

 The pH value of a soil is an indication of soil reaction i.e.acidic,neutral or alkaline. The nutrients availability is governed by soil reaction. Thus, pH value gives an idea about the availability of nutrients to plants.

**Principle**

 The pH is usually measured by pH meter, in which the potential of hydrogen ions indicating electrode (glass electrode) is measured potentiometrically against colonel saturated reference electrode which also serve as salt bridge. Now a days, most of the pH metre have single combined electrode. Before measuring the pH of soil, the instruments has to be calibrated with standard buffer solution of known pH. Since, the pH is also affected by the temperature corrections knob.

**Apparatus**

 pH meter, balance, beaker, measuring cylinder, spatula, glass rod.

**Reagents**

 Standard buffer solution. These may be of pH 4.0, 7.0 and 9.2 are prepared by dissolving one standard buffer tablet in 100 ml distilled water.It is necessary to prepare fresh buffer solution after few days. In absence of buffer tablet, a 0.05 *M* potassium hydrogen phthlalate solution can be used which gives pH of 4.0 (Dissolve 10.21 g of A.R. grade potassium hydrogen phthalate in distilled water and dilute to 1 litre. Add 1 ml of chloroform or a crystal of thymol per litre as a preservative).

**Procedure**

1. **Soil to water suspension (ratio of 1:2.5)**

 Take 20 g of soil in 100 ml beaker and add 50 ml of distilled water in it. The suspension is stir with glass rod at a regular intervals for 30 minutes. Calibrated the pH meter with pH butter solution and determine the pH by immersing electrode in suspension. For soil containing high salts, the pH should be determined by using 0.01 M calcium chloride solution. (Dissolved 0.110 g of CaCl2 in water and dilute to 1 litre).

1. **Saturated soil paste**

 Add small amounts of distilled water to 250 g of air dried soil. Stir the mixture with a spatula. At saturation, the soil paste glisten and flow slightly when the container is tapped it slide freely and ensure cleanly off the spatula. After mixing, allowed the sample to stand for an hour. If the paste has stiffened markedly or lost its glistening add more water or if free water has collected on the surface of the paste, add additional weighed quantity of dry soil and mix it again. Then insert the electrode carefully in the paste and measure the pH.

1. **Saturation extract**

 The soil is extracted using vacuum extractor and the pH is measured in the saturation extract.

**Precaution in the use of pH meter**

1. The electrode should not be allowed to remain in the test solution or suspension, for a longer period than necessary.
2. Immediate after testing the electrode should be washed off with a gentle stream of distilled water.
3. For storage, after cleaning the electrode are suspension in distilled water and the system is protected from evaporation.
4. The electrode, while not in use, should be immersed in distilled water to avoid their drying.

**Interpretation**

|  |  |
| --- | --- |
| **Soil pH**  | **Soil rating** |
| <5.0 | Strongly Acidic |
| 5.1-6-5 | Slightly Acidic |
| 6.6-7.5 | Neutral |
| 7.6-8.0 | Mild alkaline |
| >8.0 |  Strongly alkaline  |

**Experiment 3: Determination of Electrical Conductivity in soils**

Electrical conductivity is the reciprocal of resistance of a conductor. The amount of soluble salts in a sample is expressed in terms of the electrical conductivity (EC) and measured by a conductivity meter. The instrument consists of an AC Solu Bridge or electrical resistance bridge and conductivity cell having electrodes coated with platinum black. The instrument is also available as an already calibrated assembly (Solu bridge) for representing the conductivity of solutions in dS m-1 (deci Siemen per meter) at 25°C.

**Principle**

A simle Wheatstone Bridge Circuit is used to measure EC by null method. The bridge consists of two known and fixed resistance r1, r2, one variable-standard resistance r4 and the unknown r3. The variable resistance r4 is adjusted until a minimum or zero current flows through the AC galvanometer. At equilibrium:

 **r1 r3 r1**

**--------- = --------- or = r3 × ------------ × r4**

 **r2 r4 r2**

Since conductivity is reciprocal of receptivity, it is measured with the help of r3.

**Apparatus**

Conductivity meter, weighing balance, beakers, measuring cylinder, glass rod

**Reagent**

**0.01*N* potassium chloride solution:** Dry a small quantity of AR potassium chloride at 60°C for 2 hours in a hot air oven. Weigh 0.7456 g of it and dissolve in freshly prepare distilled water and make to one litre. This solution gives an electrical conductivity of 1.4118dS m-1 at 25°C.

**Procedure**

1. Transfer 20 g of soil sample in a 100 mL beaker.
2. Add 40 mL of distilled water and shake intermittently for 1 hour on a shaker.
3. Allow to stand until clear supernatant liquid is obtained. Alternatively, the clear extract after the pH measurement can also be used for EC measurement.
4. Calibrate the conductivity bridge with the help of standard KCI solution and determine the cell constant.
5. Determine the conductivity of the supernatant liquid with the help of conductivity meter.

 1.4118 dS m-1

Cell constant = ----------------------------------------------------

 Observed conductivity of KCl solution

**Calculations**

 Electrical Conductivity (dS m-1) = Observed value of EC × cell constant × temperature

 factor (to express result at 25°C)

**Precautions**

1. Soil suspension should be allowed to stand for a sufficient time so as to obtain a clear supernatant solution
2. The instrument must be set at the temperature of the test solution.
3. The electrodes should be completely immersed in the test solution to get an accurate reading.
4. The electrodes must be washed with distilled water and cleaned with filter paper after measuring the conductivity of every sample.
5. When not in use, the electrodes should be kept dipping in distilled water, otherwise, deposition of hydrogen on them will reduce their efficiency.

Note:

Even if the scale is marked to read directly, as in most of the conductivity meters, it is necessary to check/calibrate the instrument with the standard KCI solution.

**Interpretation**

**EC (**dS m-1**) Soil Category**

<1 Normal-suitable of all crops

 1-2 Critical for salt sensitive crops

 2-3 Critical for salt tolerant crops

 >3 Injurious to most crops

**Experiment 4: Determination of Organic Carbon in soils**

Carbon is the chief constituent of soil organic matter and the estimation of organic matter is based on organic carbon. The soil organic carbon (SOC) and inorganic carbon as carbonate minerals associated with soil organic matter. Soil organic matter with a fraction of the soil that is made up of decomposed plant and animal materials as well as microbial organisms, but does not include fresh and undecomposed plant materials such as straw and litter lying on the soil surface. Soil carbon can also be present in inorganic form (lime or carbonates) in some soils in the drier areas. The SOC tends to be concentrated in the top soil. The top soil (surface soil) ranges from 0.5% to 3.0% organic carbon for most upland soils and less than 0.5% are most limited to hot and drier areas. There is also a close relationship between carbon and nitrogen in the soils. Most organic matter average about 5% nitrogen so that the N:C ratio is 1:11.6. Therefore, by multiplying the soil organic matter percentage by 0.05 an approximate value for the soil nitrogen, percentage is obtained.

**Methods**

The soil organic matter is estimated from the organic carbon which can be determined by the following methods:

1. Dry combustion method

2. Wet combustion method

**Dry combustion method**

**Principle**

It is a gravimetric method. The soil is treated with sulphurous acid to destroy CaCO3, is ignited in silica tubes which results in evolution of CO2. The CO2thus evolve is, then absorbed in a weighed soda-lime tube and the amount of CO2 produced is found by differences between the initial and final weight of the soda lime tube. From the amount of soil taken and amount of CO2 evolved, content of organic carbon and hence organic matter is calculated.

 This method, though, is more accurate but a tedious and time consuming. In addition to this, the carbonate removal is more difficult. Very finely ground sample is needed for this purpose. With this method only a limited number of samples can be analyzed in a day.

**Wet combustion method**

This is a rapid and fairly method. A large numberof samples can be analyzed in a day. Here the presence of CaCO3 does not affect the determination. Most commonly used wet combustion method is Walkley and Black (1934) rapid titration method.

**Principle**

 The organic matter in the soil gets oxidized by with a mixture of potassium dichromate and concentrated H2SO4, utilizing the heat of dilution of H2SO4. The excess of potassium dichromate, not reduced by the organic matter of the soil, is determined by titration using ferrous ammonium sulphate (FAS) solution in the presence of phosphoric acid using diphenylamine as indicator.

**Reactions**

(i) The oxidation of carbon

K2Cr2O7 + H2SO4 K2SO4 + Cr2(SO4)3 + 4H2O + 3O-[×2]

3C + 6O- 3CO2

2K2Cr2O7 + 8 H2SO4 + 3 C 2 K2SO4 + 2 Cr2(SO4)3 + 8 H2O + 3CO2

(ii) During Titration

FeSO4(NH4)2SO4.6H2O FeSO4 + (NH4)2 SO4 + 6H2O [×2]

2FeSO4  + H2SO4 + [O-] Fe2(SO4)3 + H2O

2FeSO4 (NH4)2SO4.12H2O + H2SO4 + O- 2(NH4)2 SO4 + Fe2(SO4)3 + 13H2O

(iii) The action of diphenylamine indicator

 +O +O

2C6H5NHC6H5 2(C6H5NHC6H4) C6H5N-C6H4C6H4N-C6H5

Diphenylamine -H2O -H2O Diphenyl benzidine (violet)

**Apparatus**

Conical flasks, pipette, burette, measuring cylinder, weighing balance

**Reagents**

1. **1N potassium dichromate:** Dissolve 49.04 g of AR grade K2Cr2O7 in about 500 mL of distilled water and make the volume to one litre
2. **Conc.Sulphuric acid**
3. **0.5N Ferrous ammonium sulphate:** Dissolve 196g of ferrous ammonium sulphate in distilled water, add 20 mL of conc. H2SO4 and make volume to one litre. The ferrous ammonium sulphate should be from a fresh lot and light green in colour. Yellowing of the salt indicates its oxidation.
4. **Diphenylamine indicator:** Dissolve 0.5 g of the dye in a mixture of 20 mL of distilled water and 100 mL of conc. H2SO4.
5. **Orthophosphoric acid (85%) or sodium fluoride**

**Procedure**

1. Weigh 1g of 0.2 mm soil sample into 500 mL dry conical flask of borosilicate glass.
2. Add 10 mL of 1*N* K2Cr2O7 and 20 mL of conc. H2SO4 with little swirling during addition
3. Leave the flask for 30 minutes without disturbing so as to cool the contents and to make the reaction complete.
4. Add slowly 200 mL of distilled water and 10 mL of orthophosphoric acid or 0.5 g NaF.
5. Add 1 mL of diphenylamine indicator, which will give a deep violet colour of the suspension will appear.
6. The contents are titrated with 0.5 *N* ferrous ammonium sulphate solution in 50 mL burette till the colour changes from violet to bright green colour starts appearing.
7. Note the volume of the ferrous ammonium sulphate solution used in titration and calculate the results as given below (If the titre value is <6, repeat taking 0.2 to 0.5 g of soil sample).
8. Simultaneously, a blank is to be run without soil.

**Calculations**

 **(X-Y) × 0.003 × 100**

**Organic carbon in soil = ------------------------------- = Z (%)**

 **2 × W**

Where,

 W = Weight of soil taken (g)

 X = Volume of 0.5 *N* ferrous ammonium sulphate used for the blank titration

 Y =Volume of 0.5 *N* ferrous ammonium sulphate used for titrating the excess K2Cr2O7

 2H2Cr2O7+ 3C + 6H2SO4 = 3Cr2(SO4)3+ 3CO2 + 8H2O

 Thus, 2HCr2O7 or 2K2Cr2O7$≡$ 3C

 Or 588g of K2Cr2O7$≡$ 36 g of C

 Or 12 litres of 1*N* K2Cr2O7$≡$ 36 g of C

 Or 1 mL of 1N K2Cr2O7$≡$ 0.003 g of C

**Organic carbon in soil (%) = Z × 1.3 = R**

There is incomplete oxidation of organic matter in this procedure. The organic matter is multiplied by 1.3 on the assumption that there is 77% recovery.

**OC (g kg-1) = OC content (%) × 10**

**Organic matter in soil**

By assuming that organic matter contains 58% carbon, thus the organic matter content in the soil will be calculated as under:

= R × 100/58

 = 1.724

**Organic matter in soil (%) = R × 1.724**

**Interpretation**

**Organic C (g kg-1) Soil Rating**

< 5.0 Low

5.0 to 7.5 Medium

> 7.5 High

**References**

Walkley, A.J. and Black, C.A. (1934). Estimation of soil organic carbon by chromic acid titration method. Soil Sci., 37: 29-38.

**Experiment 5: Determination of available Nitrogen in soils**

 The major part (more than 90%) of soil nitrogen exists in complete combination in the organic matter (humus) fractions. It becomes available to crop after breakdown so simple form followed by mineralization. The chemical method for determination of soil determination of nitrogen availability involved either direct or indirect of total nitrogen and measurements of a fraction of easily hydrolysable nitrogen by employing solution of dilute acid or alkali. Distillation of alkaline potassium permanganate solution has often been adopting for estimate for oxidisable and reactive form of soil nitrogen. Alkaline permanganate method however is the quickest of all other methods for the estimation of available nitrogen and has been found to work well even in Indian soil.

 The available of nitrogen in soil is determined by alkaline permanganate methods as per procedure suggested by Subbiah and Asija (1956).

**Principle**

 A known weight of the soil is mixed with alkaline potassium permanganate (KMnO4) solution and distilled. The organic matter present in soil is oxidized by the nascent oxygen liberated by potassium permanganate, in the presence of sodium hydroxide and the released ammonia is condensed and adsorbed in known volume of a boric acid containing mix indicator to form ammonium borate, the excess of which is titrated with a standard sulphuric acid.

**Reaction**

**(i) Distillation**

 Alkaline

 2KMnO4 +H2O 2MnO4+ 2KOH + 3O-

 Medium (Nascent oxygen)

 Oxidation

 R. CHNH2COOH + O- R. CO.COOH + NH3

 (organic N fraction) (Ammonia)

Distillation

 NH3 + H2O NH4OH

 (Ammonium hydroxide)

 Absorption

3NH4OH + H3BO3 (NH4)3BO3 + 3H2O

(Boric acid) (Ammonium borate)

 (Green Colour)

**(ii)Titration**

 2(NH4)3BO3 + 3H2SO4 2(NH4)3SO4 + 2H3BO3 (Pink colour)

**Apparatus**

1. Automatic Nitrogen DistillationSystem

The said instrument is use for determination of available nitrogen in soil. It consist of the following:

* Automatic distillation system: It is fully automatic distillation system with programmable auto run digital features, with automatic dilution, addition of NaOH, KMnO4 and boric acid both modes (auto and manual) are available for distillation and reagents addition

2.Electronic balance

3.Burette

4.Conical flash

**Reagents**

* 1. 0.32% potassium permangenate (KMnO4) solution
	2. 2.5% sodium hydroxide (NaOH)
	3. 2% boric acid solution containing 20 – 25 ml of mixed indicator / litre
	4. Mixed indicator: Dissolved 0.066 g Methyl red and 0.099 g of bromocressol green dissolved in 100 ml of 95 % of alcohol
	5. 0.02 *N* standard sulphuric acid (H2SO4) solution.

**Procedure**

1. Weigh 5 g of soil sample and transfer it to the digestion tube.
2. Load the tube in distillation unit and other sides of hose keep 20 ml of 2% boric acid containing mixed indicator in 250 ml conical flask
3. 25 ml each of potassium permanganate (0.32%) and sodium hydroxide (2.5%) solutions is automatically added by distillation unit.
4. The sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 20 ml of 2% boric acid containing mixed indicator solution kept in a 250 ml conical flask.
5. With the absorption of ammonia in boric acid, the pinkish colour turns to green and nearly 150 ml of distillate is collected in about 10 minutes.
6. The green colour distillate is titrating with 0.02 *N*sulphuric acid, the end point colour changes from green to original shade (pinkish colour).
7. Simultaneously, a blank is to be run without soil.
8. Note the volume of 0.02 *N*sulphuric acid and calculate the amount ofavailable nitrogen present in soil.

**Calculations**

Available soil N (kg ha-1)

 R (STR - BTR) x Normality of acid x Atomic weight of nitrogen x Weight of one hectare of soil (0- 15 cm soil depth)

= -----------------------------------------------------------------------------------------------------------------------------------------------------------

 Weight of soil (g) x 1000

 R x 0.02 x 14 x 2.24 x 106

= -----------------------------------------------

 5 x 1000

Factor = R x 125.44

Where,

 STR = Sample titre reading (ml)

 BTR= Blank titre reading (ml)

 R= Final reading (mL) obtained from subtraction of sample titre reading to blank titre reading

**Interpretation**

 Available soil (kg ha-1) Soil rating

 <250 Low

 250 – 400 Medium

 >400 High

**References**

Subbiah, B.V. and Asija, G. L. (1956). A rapid procedure for the estimation of nitrogen in soils. Current. Science 25: 259-260

**Experiment 6: Determination of Available Phosphorous in Soils**

Available phosphorus refers to that fraction of this element, which can be easily absorbed from the soil by plant roots. Phosphorus occurs in soils both in organic and inorganic forms. Plant takes phosphorus as H2PO4- and HPO42- ions. The major source of these phosphate ions is the inorganic forms. Iron phosphate and aluminium phosphate in acid soils and calcium phosphate in calcareous soils act as the source of inorganic phosphorus. All these inorganic forms of phosphorus are in dynamic equilibrium with water-soluble phosphorous in soils. So, it is obvious that the method that is employed for the determination of available phosphorus in soil must include predominantly these inorganic forms, in addition to the water-soluble phosphorus.

The chemical determination of available phosphorus involves two phases:

1. Extraction of soil

2. Analysis of extract

There are several laboratory methodologies by which the availability of phosphorus in soils is determined. Soil type and soil pH are the main factors which determine which methodology should be used. Various extracting solutions have been suggested and accordingly the relative amount of phosphorus brought into solution by extraction differs depending partly upon the amount present in the soil and partly on the solubility in various solvents. The available phosphorus is extracted with 0.5 M NaHCO3(Olsen's method) is used for neutral to alkaline soil and colour developed the filtrate of P by ascorbic acid reductant method (Watanabe and Olsen, 1965) as described below:

**0.5 M NaHCO3 (Olsen's et al., 1954)**

**Principle**

Soil is shaken with the 0.5 M NaHCO3 solution for half an hour in the presence of Darco G-60 and the extract is obtained by filtering the suspension. The phosphorus in the extract is treated with ammonium molybdate which results in the formation of a hetropoly complex known as phosphomolybdate (yellow-coloured). Then the phosphomolybdate is reduced by the use of ascorbic acid (a reducing agent) give blue colour. The intensity of blue colour, which is proportional to the concentration of phosphate is measured with a spectrophotometer at a wavelength of 760 nm. The extractant used in this method depress the activity of Ca by precipitating it as CaCO3 in neutral, alkaline and calcareous soils and thus increases the concentration of phosphorus in solution.

**Reactions**

**(D) Extraction**

Ca3(PO4)2 + 6 NaHCO3 3 CACO3 + 2H3PO4 + 3 Na2CO3

 **(ii) Color development**

(NH4)6MO70244H20 + 6 HCI 7H2MoO4 + 6NH4Cl

(Ammonium molybdate) (Molybdenic acid)

H3PO4  + 12H2MoO4 H3P(Mo3O10)4 + 12H2O

(Phosphoric acid) (phosphor molybdate)

 Yellowed-coloured complex

Phosphomolybdate + ascorbic acid Reduced phosphomolybdate

 (Blue colour complex)

**Apparatus**

Spectrophotometer, electrical balance, mechanical shaker, pipette, conical flask, volumetric flask, funnel, Whatman No. 1 filter paper.

**Reagents**

1. 0.5 M Sodium bicarbonate (NaHCO3) solution: Dissolve 42 g of NaHCO3 in distilled water and make up the volume to 1 litre after adjusting the pH to 8.5 by adding small quantity of NaOH or HI if required.
2. Darco G-60 (Activated charcoal).
3. Ammonium molybdate (Solution A): Dissolve 12 g of AR grade ammonium molybdate in about 300 ml warm distilled water. In 100 ml of distilled water, dissolve 0.291 g of antimony potassium tartrate separately. Mix both of the solutions and add 148 ml concentrate sulphuric acid and make the final volume to 2 litres with distilled water and store the solution in dark coloured bottle.
4. Ascorbic acid solution (Solution B): Dissolved 1.056 g of ascorbic acid in 200 ml of solution A (This should be prepared fresh as and when required)
5. 5N Sulphuric acid (H2SO4): Dilute 148 ml concentrated H2SO4 to 1 litre.
6. Standard phosphate solution: Dissolve 0.439 g of AR grade of potassium dihydrogen orthophosphate (KH2PO4) in a 1 litre distilled water. Add to it 25 ml of 7 N H2SO4and make volume to 1 litre. This gives 100 ppm stock solution of phosphorus.
7. Working phosphate solution: From 100 ppm P solution prepares a 2 ppm solution by 50 times of dilution of the stock solution and get a final P concentration range varying from 0.1 to 1.0 ppm.

**Procedure**

1. Weigh 2 g of soil and transfer to a 100 mL conical Mask.
2. Add a pinch of Darco G-60 and 40 ml of 0.5 M NaHCO3 solution.
3. The flasks shake on an electric shaker at a constant speed for 30 minutes.
4. The contents filtered immediately through dry filter paper (Whatman No. 1) in to clean and dry beakers.
5. Pipette 5 ml of the filtrate in a 25 mL volumetric flask. Add 0.5 ml of 5 N H2SO4 and shake the flask until evaluation of CO2 stops.
6. After acidify the aliquot, add distilled water and diluted upto 20 ml.
7. After dilution, add 4 ml of solution B (Ascorbic acid solution) to it and make the volume upto the mark (25 ml) by adding distilled water and mix the contents of the flask.
8. After waiting 10 minutes, the intensity of the blue colour developed is measure using spectrophotometer at 760 nm wavelength and note the absorbance reading.
9. Similarly, prepare a blank without taking soil sample.

**Bray's P-1 (Bray and Kurtz, 1945)**

***Instruments***

1. Mechanical shaker

2. Colorimeter or Spectrophotometer

***Reagents***

1**. Bray's P-1 extractant:** Dissolve 1.110 g of AR grade ammonium fluoride in one litre of 0.025/N HCI.

2**. 1.5% Dickman and Bray's reagent:** Dissolve 15 g of AR grade ammonium molybdate in 300 mL. of warm water, cool and add exact 350 mL of 10N HCI. Make the volume to one litre.

3**. 40% SnCI2 stock solution**: Weigh 10 g pure stannous chloride in a 100 mL glass beaker. Add 25 mL of conc. HCl and dissolve by heating. Cool, transfer to an amber coloured bottle and store in dark after adding a small piece of Zn metal (AR grade) to prevent oxidation. From this, prepare a dilute SnCl2 solution (0.5 mL. diluted to 66 mL) immediately before use.

4. **Standard stock solution:** Weigh 0.439 g of AR grade KH2PO4 dried in oven at 60 °C for 1 hour in a one litre beaker, add about 500 mL of distilled water and dissolve. Add 25 mL of approx. 7N H2SO4 and make the volume to one litre. This is 100 mg PL-1 solution.

5. **Standard working solution**: Dilute a suitable volume of 100 mg PL-150 times to get 2 mg P L-1 solution.

**Procedure**

1. Weigh 5 g of soil sample in a 150 mL conical flask.

2. Add 50 mL of Bray's P-1 extractant and shake for 5 minutes.

3. Filter through Whatman No.1 filter paper quickly so as to collect the filtrate within 10 minutes.

4. Transfer 5 mL aliquot into a 25 mL volumetric flask.

5. Add 5 mL of ammonium molybdate solution, shake a little and dilute to about 22 mL.

6. Add 1 mL of diluted SnCl2(0.5 mL diluted to 66 mL), mix by shaking a little, and make up the

volume.

7. Run a blank without soil under identical conditions.

8. Measure the intensity of the blue colour developed, using 660 nm wavelength (red filter).

**Preparation of standard curve**

1. The standard curve shows the relationship between the concentration of an element in the solution and intensity of its colour. This is used to calculate the amount of that element in the unknown samples.
2. To prepare the standard curve pipette out 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of 2 ppm working solution of P in different seven 25 ml volumetric flasks. The proceed to develop colour like the test sample.
3. The intensity of blue colour is read on spectrophotometer at 760 nm wavelengths after 10 minutes and note the absorbance reading,

1. Plot the graph of P concentration versus absorbance readings on a simple/ semi log graph paper.

**Calculations**

 **Q × V × 2.24 × 106 Q × V × 2.24**

**Available P (kg ha-1) = ………………………………… = ………………………………..**

 **A × S× 106 A × S**

**Where,**

Q = Quantity of P in read on X-axis against a sample reading

 V = Volume of extracting reagent used (mL)

 A = Volume of aliquot used for colour development (mL), and

 S = Weight of soil sample (g)

Thus,

 Bray’s P (kg ha-1) = Q **×**4.48

Olsen’s P(kg ha-1) = Q **×**8.96

**Available P2O5 (kg ha-1) in soil** = Q × 2.29

**Precautions**

1. Sufficient time should be given to the instrument for warming up.
2. The test tube must be cleaned from outside with the help of a filter paper so as to remove any drop of the solution sticking to its sides.
3. While inserting the test tube in the cuvette, care should be taken that the white line on the test tube coincides with the red line on the cuvette.
4. Every time when a new sample is to be tested, the test tube should be rinsed with the same and then and only then the test tube should be filled with it and inserted in the cuvette.
5. Test tube should be handled from the top.
6. Filtrate should be colourless.
7. Ascorbic acid solution should be prepared afresh.
8. If the colour intensity is too high, take a smaller amount of filtrate and redevelop the colour. Do not dilute the coloured solution.

**Interpretation**

**Soil rating Available P (kg ha)**

Very low : Less than 5

Low : 5 - 10

Medium : 10 – 20

High : 20 - 40

Very high : More than 40

**References**

Olsen, S. R., Cole, C. V., Watanabe, F. S. and Dean. L. A. (1954). Estimation of available phosphorus in soil by extraction with sodium bicarbonate. U. S. Deptt. of Agri. Circular 939.Washington D.C. USA: U.S. Govt. Printing office. Pp 19.

Watanabe, F. S. and Olsen, S. R. (1965). Test of ascorbic acid method for determining phosphorus in was and sodium bicarbonate extracts of soil. Proc. Soil Sci. Soc. Am.,29 : 677-78.

**Experiment 7: Determination of available Potassium in soil**

Potassium is essential for photosynthesis, for protein synthesis, for starch formation and for translocation of sugars. It also acts as an activator of enzymes. About 90-98% of the total potassium in soils is present in minerals, such as potash feldspar, muscovite and biotite micas. Potassium from these minerals is released slowly by weathering process and usually is not of much significance to meet immediate crop requirement. However, cumulative contribution of this over a period of time is of considerable importance. The readily available K constitute about1-2% of total K in mineral soil. It consists of soil solution and exchangeable K absorbed on soil colloidal surfaces.

The, neutral normal ammonium acetate solution which extracts both exchangeable and water soluble K in most commonly used for determination of available potassium in soils.

**Principle**

Ammonium acetate is used to extract exchangeable K in soil as ionic diameter ofNH4+ is closely similar to that of K+ in soil and is readily replaced by NH4+. Potassium in the extract is atomized into blue flame of a flame photometer so that it gets excited on gaining energy and emits radiation of certain wave length in proportion to the concentration of K ions. The principle underlying this is that a large number of elements when excited in a flame, emit radiation of characteristic wave length. The excitation causes one of the outer electrons of neutral atoms to move to an outer orbit of higher energy level or the atoms may be excited sufficiently to lose an electron completely from the attractive force of the nucleus where excited atom return to lower energy level, light at characteristic wave length is emitted. Excited atoms or ions give line radiation at very definite wave length and thus K gives at 404.4 mµ and 767 mµ. The flame photometer employs a relatively low temperature excitation and measures with a photocell the emission intensity which is proportional to concentration in selected wave length (767 mµ) and for this red filter is used.

**Apparatus**

Flame photometer, electronic balance, mechanical shaker, funnels, beaker, pipette, conical flask, volumetric flask, Whatman No. 1 filter paper

**Reagents**

1. Neutral normal ammonium acetate solution: Dissolve 77.08 g of ammonium acetate(CH3COONH4) in distilled water and make the volume to 1 litre. Adjust the pH of solution to 7.0 with glacial acetic acid or NH4OH.
2. Standard K solution: A stock solution of 1000 ppm K is made by dissolving1.908 g of AR grade dried potassium chloride (KCl) in distilled water and make up the volume to 1 litre. Dilute suitable volumes of stock solution to get 100 ml of working standard containing 5, 10, 15, 20, 25 and 40 ppm K.

**Procedure**

1. Weigh 5 g of soil and transfer in a 100 ml conical flask.
2. Add to it 25 ml of neutral normal ammonium acetate solution and shake the content of flask for 5 minutes.
3. Filter the content through Whatman No. 1 filter paper.
4. Feed this solution to the atomizer of the flame photometer after calibration and record the reading on the screen.

**Calculations**

 Flame photometer reading in ppm (R) × Volume of the

extractant added (mL) × weight of 1 ha soil (0-15 cm soil depth)

Available K in soil (kg ha-1) =---------------------------------------------------------------------------------------

Weight of soil taken (g)

Factor =R × 11.20

**Precautions**

* The air pressure should neither be below 0.4 kgcm-2nor it should exceed 0.75kgcm-2,
* The gas and air pressure should be constant.
* The flame should be soot-free and blue with all the burner cones visible clearly.
* While starting the instrument turn on the air supply before turning on the fuel supply.
* When extinguishing the Name always turn off the fuel before turning off the air.
* The flame photometer should be warmed up for 10-15 minutes before use with continuous water feeding.
* When all the test samples have been analyzed, the atomizer must be fed with distilled water continuously for about 2 minutes.
* These should not be any turbidity or suspended particles in extract, it will chock the capillary feeding tube
* If sample reading goes beyond 100 then dilute the extract.

**Interpretation**

**Available K (kg ha-1) Soil rating**

Less than 200 Very low

200 – 250 Low

250 – 400 Medium

400 – 600 High

More than 600 Very high

**Experiment 8: Estimation of exchangeable Calcium and Magnesium in soils**

Exchangeable Ca and Mg are normally determined in ammonium acetate extracts of soils (obtained as discusses above K) by direct titration with EDTA or by using the AAS. Both these methods are described here.

**EDTA titration method**

The presence ammonium acetate does not interfere with the EDTA titration method if carried out by the procedure suggested below (Hesse 1971). The amount of organic matter dissolved is usually too small to affect the colour change of the organic of the indicator. This procedure permits the determination of Ca and Mg in the same solution.

**Reagents**

1. Standard calcium solution: Weigh 0.5005 g of CaCO3 dried at 150°C into 1 liter of volumetric flask. Add 200 ml of water and then 150 ml of 1N HCl slowly and with shaking. Dilute the solution to 1 liter. This solution is 0.01N in Ca.
2. EDTA solution: Dissolve 2.0 g of ethylenediamine tetra acetic acid (di-Na salt) in water and dilute to 1litre.
3. Sodium hydroxide aqueous solution: 10% w/v.
4. Buffer solution: Dissolve 67.5g NH4Cl in 400ml of water, add 570 ml of conc. NH4OH and dilute to 1 litre.
5. Hydroxylamine -hydrochloride aqueous solution: 5% w/v. prepare fresh each week.
6. Potassium hexacyanoferrate (II) aqueous solution (potassium ferrocyanide) 4% w/v.
7. Potassium cyanide aqueous solution:1% w/v.
8. Triethanolamine: Commercial reagent.
9. Calcon solution: Dissolve 0.2 g reagent in 50 ml methanol. Prepare fresh every two weeks.
10. Eriochrome Black T solution: Dissolve 0.2 g reagent in 50ml methanol. Prepare fresh every two weeks.

**Procedure**

**Standardization of EDTA solution**

1. Pipette 5 mL of standard calcium solution into a graduated, tall-form, 100 mL beaker.
2. Dilute to 10 mL and add 15 mL of ammonium chloride-ammonium hydroxide buffer solution.
3. Add 10 drops each of potassium cyanide, hydroxylamine-hydrochloride, potassium hexacyanoferrate (II), triethanolamine and Erichrome Black T solution.
4. Place the beaker on a magnetic stirring plate and start stirring.
5. Prepare a blank solution in exactly the same manner, taking 5 mL of water instead of calcium solution.
6. Usually the blank solution will be blue in colour, but if not, it should be titrated with EDTA solution until blue and the blank titre value noted.
7. Keep the blue blank solution alongside the standard calcium solution and titrate the standard with EDTA solution, stirring all the time to a permanent blue colour matching the blank.
8. Dilute the blank with water now and again to equalize the volumes of the two solutions as the titration proceeds.
9. Repeat the standardization using Calcon as indicator. Add to the diluted calcium solution 10 drops each of potassium cyanide, hydroxylamine hydroxide, and triethanolamine solutions.
10. Add 2.5 mL NaOH solution and 1 mL of Calcon solution.
11. Prepare a reagent blank and titrate both solutions with EDTA solution until blue.

**Determination of Ca in the NH4OAc extract**

1. Pipette an aliquot of the extract containing up to 3 mg Ca into a 100 mL tall form beaker and dilute to 10 mL (Note: For soils containing less than 10 m.e. Ca/100 g or solutions containing less than 1 mg Ca/10 mL, it is necessary to take larger aliquots in a 250 mL beaker).
2. Add 10 drops each of potassium cyanide, hydroxylamine-hydrochloride and triethanolamine solutions.
3. Add 2.5 mL NaOH solution and 1 mL of Calcon solution.
4. Titrate with EDTA until blue.

**Determination of Ca + Mg**

1. Pipette an aliquot containing up to about 3 mg (Ca+Mg) into a beaker.
2. Dilute to 10 mL, add 15 mL of ammonium chloride-hydroxide buffer solution and then 10 drops each of potassium cyanide, hydroxylamine-hydrochloride, potassium hexacyanoferrate (II) and triethanolamine solutions while gently warming the solution on the magnetic stirrer.
3. Continue warming for 3 minutes when all the reagents have been added.
4. Cool and add 10 drops of Erichrome Black T solution and titrate with EDTA.

**Determination of Mg**

Mg is calculated from the difference between the Ca+Mg and the Ca determinations.

**Calculation**

 T × Normality of EDTA × 1000

Ca or (Ca + Mg), meq/litre = --------------------------------------------------

 Aliquot (mL) taken

Where T = Volume in mL of standard EDTA used in titration

Or

 100 Extract volume (mL)

Ca or (Ca +Mg) meq/100 g soil = --------------------- × --------------------------------× meq Ca or (Ca + Mg)/ litre

Soil weight (g) 1000

**Experiment 9: Estimation of available Sulphur in soils**

Sulphur occurs in different form in soil viz, sulphites, sulphates, sulphides and inorganic compounds. Plants absorb S in the form of sulphate ion (SO4-2) and its conc. Inplants ranged from 0.1 to 0.5 %. The S ion is primarily adsorbed by clays and Fe and Al oxide. Among the different extractants, CaCl2 (0.15%) was found to be the best as suggested by Williams and Steinbergs (1959). If the amount of available S in soil is too low than the critical level of 10 mg kg-1 CaCl2 extractable SO4-S, then yield is jeopardised, but increasing reserves in soils to very high levels is an unnecessary expense. Thus, the analysis of sulphur is an aid to overcome its deficiency, which is gradually becoming widespread in different soils of the country due to high analysis S-free fertilizers coupled with intensive cropping. higher crop yields and higher S removals.

**Principle**

When the soil solution is shaken with CaCl2 (0.15 %), the chloride ions displace the adsorbed sulphate during extraction. The filtrate is analysed for sulphur by turbidimetry method as outlined by Chesnin and Yien (1951), in which turbidity produced due to the precipitation of SO4-2 as BaSO4when excess BaCl2.2H20 is added to a solution is measured on a spectrophotometer at a wavelength of 420 nm or corresponding to blue filter. Gumacacia is usually included to stabilize the fine suspension of BaSO4

**Glassware and Apparatus required**

50 ml Erlenmeyer flask, volumetric flask, pipette, Balance, mechanical shaker, conical flask, measuring cylinder, beaker, funnel, burette, and spectrophotometer

**Reagents**

1. Calcium chloride (0.15 %): Dissolve 1.4702 g of CaCl2.2H20 in distilled water and make the volume to 1 litre.
2. Salt buffer solution: Dissolve 40 magnesium chloride and 4.1 g of potassium nitrate and 28 ml ethanol per litre and dilute the solution to 1 litre with distilled water.
3. Gum acacia solution: Dissolve 0.5 g gum acacia in a mixture of 50 ml acetic acid and 50 ml of distilled water Store the solution in a refrigerator to avoid microbial growth.
4. 6N HCL: Dissolve 500 ml conc. HCI in 500 ml distilled water.
5. BaCl2.2 H2O crystals: 30-60 mesh
6. Activated charcoal free from sulphur
7. Standard sulphate-S stock solution (100 ppm): Dissolve 0.5434 g of AR grade K2SO4 in distilled water and dilute to 1 litre.
8. Working solution (40 ppm): Pipette out 40 ml of 100 ppm S solution in to 100 ml volumetric flask and mark up to 100 ml.

**Preparation of standard curve**

Pipette out 0, 1.0, 2.0, 3.0, 4.0, 5.0 ml of 40 ppm sulphur solution into 50 ml Erlenmeyer flasks to prepare the working standard of 0, 40, 80, 120, 160 and 200 µg S ml-1respectively. Add 1 ml each of salt buffer solution, 6N HCI and gum acacia. Mix the content and add (0.5g) or a pinch of BaCl2crystals. Allow the solution for one minute and then swirl the content gently until the barium chloride crystals dissolved. After 10 minutes, the turbidity developed in the standards is measured in a spectrophotometer at a wavelength of420 nm.

**Extraction of SO4-S**

1. Weigh 5 g air dry soil (2 mm) in 50 ml Erlenmeyer Masks.
2. Add 25 ml of 0.15% calcium chloride dihydrate solution and 0.5 g activated charcoal powder free from sulphur.
3. Shake for 30 minute on a reciprocal shaker (180 oscillation per minute).
4. Filter the suspension through Whatman No. 42.

**Measurement of SO4-S**

1. Pipette 10 ml aliquot of the extract in to 50 ml Erlenmeyer flasks.
2. Add 1 mi each of salt buffer solution, 6NHCI and gum acacia. If S content is more than add more quantity of gum acacia to stabilize turbidity.
3. Mix the content and add a pinch (0.5 g) of BaCl2 crystals of 30 to 60 mesh then shake vigorously to dissolve the BaCl2 and obtain a homogeneous suspension.
4. After 10 minutes, the turbidity developed is measured in a spectrophotometer at a wavelength of 420 nm.

**Calculation**

|  |  |  |
| --- | --- | --- |
| a | Weight of soil taken | = 5g |
| b | Volume of extractant added | = 25 ml |
| c | Volume of aliquot of the extract in analysis | = 10 ml |
| d | Absorbance (A) as a read from spectrophotometer | = A |
| e | S from standard curve against absorbance(factor) | = X mg kg-1 |

 factor × reading absorbance × volume of extract used

Available S (mg kg-1) = -------------------------------------------------------------------------------

 Weight of soil taken(g) × aliquot taken

Available S in kg ha-1 = (S mg kg-1) × 2.24

**Interpretation**

The available S content in in the given soil is mg kg-1 and is rated as **Deficient <10 mg kg-1** and **Sufficient >10 mg kg-1**.

**Experiment 10: Determination of available Boron in soils**

**Principles**

Boron exists in organic as well as inorganic forms in soils, and most of the B-compounds that have high availability are water soluble. Boron deficiency is encountered in calcareous and acid soil, whereas toxicity occurs in salt affected soils as well as in soils irrigated with high B water. Among the methods proposed from time to time for B determination, the most widely used one was that of Berger and Truog (1939), which involved refluxing of soil with hot water. The method was, however, time consuming and also required special reflux apparatus. Subsequently, Gupta (1967) developed a rapid and easy method wherein the soil could be extracted by boiling with water directly on a hot plate. Use of azomethine-H (John *et al.*, 1975) in place of carmine or curcumin has further simplified the determination of hot-water soluble B. Azomethine-H forms a stable coloured complex with H3BO3 at pH 5.1 in aqueous media, which retains proportional absorbance-concentration properties for several hours independent of the presence of a variety of salts.

**Hot-water Soluble Boron (Gupta, 1967)**

**Apparatus**

1.Spectrophotometer

2. Hot plate

3. Refrigerator

**Reagents**

1. Buffer solution: Dissolved 250g of ammonium acetate (NH4OAc) and 15g of ethylene diamine tetra acetic acid (EDTA disodium salt) in 400 ml of distilled water. Slowly add 125 ml of glacial acetic acid and mix thoroughly.
2. Azomethine-H reagent: Dissolve 0.45g of azomethine-H in 100 ml of 1% L-ascorbic acid solution. Store in polypropylene bottle in a refrigerator. Fresh reagent should be prepared weekly.
3. Calcium hydroxide suspension: Add 0.4g Ca(OH)2 to 100 ml distilled water.
4. 0.1 N HCl: Add 8.3 ml conc. HCl to 900 ml distilled water, mix, cool to room temperature and make up the volume to 100 ml.
5. Calcium chloride (0.01*M*): Dissolve 1.11g of anhydrous CaCl2 in 900 ml distilled water and make up the volume to 1000 ml.
6. Boron standard solutions: Dissolve 0.114g of AR grade boric acid (H3BO3) is this till water and make the volume to 1000ml. Each ml of this solutions contain20 µg of B. Dilute 0.5,1,2,3,4,5,10,20,30,40 and 50 ml of this of this stock solution to 100 ml with distilled water to have solution concentration of 0.1 ,0.2,0.4,0.6,0.8,1.0,2.0,4.0,6.0,8.0 and 10µg B ml‑1, respectively.
7. Activated charcoal

**Preparation of standard curve**

Take 1 ml of aliquot of bank and diluted B standards into a 10 ml polypropylene tube, add 2ml of buffer solution and mix. Add 2 ml of azomethine-H reagent, mix and after 30 minutes read the absorbance at 420 nm in spectrophotometer. With the help of absorbance reading of standard solution of different concentration of B the standard curve is drawn and a factor for concentration of B for 1 absorbance is calculated which is utilized to calculate B in the soils, plant or water sample.

**Procedure**

1. Weigh 20 g of air-dry (20-mesh) soil sample in 250 mL quartz or other boron-free conical flask and add 40 mL of distilled water.
2. Add 0.5 g of activated charcoal and boiled for 5 min on a hot plate, filtered immediately through Whatman No.42 filter paper.
3. Cool the contents to room tempt and transferred 1ml aliquot of blank(filtrate obtained after boiling of distilled water and activated charcoal), diluted B standard or sample filtrate into 10 or 15 ml polypropylene tubes.
4. Add 2ml of buffer solution and mix
5. Add 2ml of azomethine-H reagent, mixed and after 30 mins read the absorbance at 420 nm on a spectrophotometer.
6. Prepare a standard curve plotting B concentration (0-10µg B mL-1) on X-axis and absorbance on Y-axis
7. Refer the absorbance reading of sample aliquots to the standard curve to obtain B content of aliquot.

**Calculation**

Available B (mg kg-1 or ppm) in soil =A×2

where, A is the B content (µg) obtain from standard curve

**Precaution**

1.As far as possible avoid the use of borosilicate glass in determination and storage of reagent

2. Don’t store azomethine-H reagent for long. Prepare a fresh solution every week.

3.In order to eliminate interferences due to Al in high –Al acid soils, use tetrasodium salt of

EDTA instead of disodium salt for preparation of buffer solution.

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**Experiment 11. Determination of Zn, Cu, Fe and Mn by DTPA-CaCl2-TEA**

 **Extraction Method**

**Principle**

Diethylene Triamine Penta Acetic Acid (DTPA), a chelating agent, combines with free metal ions in solution and forms soluble complexes. Due to the reduced ionic activity in solution desorption takes place, bringing in some more ions from solid phase. DTPA offers the most favourable combination of stability constants for the simultaneous complexing of Zn, Cu, Fe and Mn. Since Fe and Zn deficiencies are frequently experienced in calcareous soils, the method is designed to avoid excessive dissolution of CaCO3 with the release of occluded micronutrients, which are normally not available to plants. This is achieved by i) the inclusion of soluble Ca++, and ii) buffering the reagent at pH 7.3 with triethanolamine (TEA) which burns cleanly during flame atomization. When the extractant is added to soil, additional Ca++ and some Mg++ enter the solution. This is largely because the protonated TEA exchanges with these ions from the exchange sites and this leads to the increased ionic concentration of Ca++ in the solution, which in turn helps in suppressing the dissolution of CaCo3. DTPA extractant has the ability to chelate Zn, Cu, Fe, and Mn in competition with Ca++, and Mg++, and unlike most other chelating agents, it applies a moderate stress to solubilize soil Fe at a pH where CaCO3 is not dissolved. Suitability of this method has been proved through excellent relationships between the test values and plant utilizable nutrients under pot and field studies conducted world over.

**(i) Determination of Available Zinc**

**Instruments**

1. Atomic absorption spectrophotometer (AAS)
2. Mechanical shaker

**Reagents**

1. **Dilute HCl:** Dilute AR grade HCl 5 times with double distilled water (DDW).
2. **DTPA extractant:** Dissolve 1.967 g of AR grade diethylene-triamine-penta acetic acid (DTPA) and 1.470 g of CaCl2.2H2O (AR grade) in about 25 mL of double distilled water (DDW) by adding 13.3 mL of triethanolamine (TEA), followed by 100 mL more of DDW. Transfer the solution to one litre volumetric flask giving 4-5 washings. Just before making up the volume, adjust pH to 7.3 with dilute HCl. This reagent has 0.005 *M*DTPA, 0.1 *M*TEA and 0.01 *M* CaCl2.2H2O.
3. **Standard stock solution ‘A’ (1000 mg Zn L-**1): Weigh exactly 1.0 g of pure Zn metal (AR grade) and dissolve it in minimum volume (about 10 mL) of dil. HCl (1:1) and make the volume to one litre.
4. **Standard solution ‘B’:** Dilute 5 mL of solution A to 100 mL to get solution B containing 50 mg Zn L-1
5. **Standard working solutions:** Dilute 0.5, 0.1, 1.5, 2.0, 2.5 and 5.0 mL portions of solution B to 50 mL to get working standards containing 0.5, 1.0, 1.5, 2.0, 2.5, and 5.0 mg Zn L-1. The working standards should be prepared in the medium of the extracting solution after every few days as these cannot be preserved for long

**Procedure**

1. Weigh 10g of soil sample in 100 mL conical flask.
2. Add 20 mL of the DTPA extractant and shake for 2 hours on a mechanical shaker.
3. Filter through Whatman No.42 filter paper, discarding first few drops. For quick filtration, Whatman No.1 filter paper can also be used if the filtrate is clear.
4. Use the filtrate for Zn measurement on AAS.
5. Feed the standard working solutions and prepare a standard curve by plotting AAS readings against Zn concentrations.

**Calculation**

A × 20

Available (DTPA-extractable) Zn in soil(mg kg-1) = ---------------- =A × 2

 10

where ‘A’ stands for the Zn concentration in aliquot as read from X-axis of standard curve against the sample reading.

**(ii) Determination of available Copper**

Available copper can be determined in the DTPA extract similar to Zn, using AAS. For this, the standard stock solution can be prepared as given below:

Accurately weighed 1.0gAR grade copper metal wire or turning and dissolve it in 50 ml of diluted HNO3 (1:1 with DDW) and finally make the volume to one litre. This is solution ‘A’ containing 1000mg CuL-1.Prepare solution ‘B’ containing50 mg CuL-1by diluting appropriate volume of solution ‘A’. Finally prepare working standard containing 0.25,0.50,1.0,1.5,2.0 and 2.5mg Cu L-1 from solution ‘b’.

**(iii) Determination of Available Iron**

Iron in the DTPA extract can also be determined with the help of AAS exactly in the same manner as Zn and Cu describe above. However, the working standard solution of Fe should be prepared for higher concentration, as the DTPA –extractable Fe content of soil is generally more than both Zn and Cu. Thus, the Fe standard may be prepared as given below

Prepare standard stock solution (solution ‘A’) by dissolving exact 1.0g of AR grade Fe in about 50 ml of 1:1 diluted HNO3 and dilute the content to 1litre with DDW. Prepare solution ‘B’ by diluting 50ml of solution ‘A’ to 500ml to get 100mg Fe L-1. Finally prepare working standard solution containing 1.0,2.0,3.0,5.0 and 10.0mg Fe L-1 by diluting appropriate volume of solution ‘b’ with the medium of extraction (DTPA in this case).

**Determination of Available Manganese**

DTPA –extractable Mn is also determined following the same technique as adopted for Zn, Cu and Fe.

For this, prepare the standard solution as follow:

Weighed 1.583g of AR grade MnO2 or 1.0 g of pure Mn metal and dissolve it in 50ml of diluted HNO3 (AR grade). Make the volume to 1L with DDW to get solution ‘A’ having a Mn concentration of 1000 mg L-1. From solution ‘A’ dilute 25 ml to 250ml with DDW to get solution ‘B’ having 1000mg Mn L-1. Finally prepare working solution of 0.5,1.0,2.0,2.5 and 5.0 mg Mn L-1 concentrations by diluting 0.5, 1.0, 2.0, 2.5 and 5.0 ml portion of solution ‘B’ 100ml.

**References**

Lindsay, W.L. and Norvell, W.A. (1978). Proc. Soil Sci. Soc. Am. **42:**421-428.

**Experiment 12: Determination of Gypsum Requirement of soil**

**Principle**

Gypsum requirement of alkali soils can be determined by treating the soil with known excess of saturated gypsum solution, and then estimating the unreacted or unutilized amount by versenate titration as suggested by Schoonover (1952). Though Ca can be estimated by other methods also, the titration method is more suitable.

**Instrument**

Mechanical shaker

**Reagents**

1. **Saturated gypsum solution:** Add 5g of chemically pure CaSO4.2H2O to one liter of distilled water. Shake vigorously for 10 minutes using a mechanical shaker and filter through Whatman no.1 filter paper.
2. **0.01 *N* CaCl2 solution:** Dissolve 0.5 g of AR grade CaCO3 in about 10mL of 0.5*N* HCl. When completely dissolved, transfer to 1liter volumetric flask and dilute to the make with distilled water. CaCl2 salt being highly hygroscopic should not be used.
3. **0.01 *N* Versenate solution:** dissolve 2.0g of pure EDTA-disodium salt and 0.05g of magnesium chloride (AR) in about 500mL of water and dilute to 1 liter. Titrate a portion of this against 0.01N CaCl2 solution to standardize.
4. **Eriochrome black T indicator:** Dissolve 0.5 g of EBT dye and 4.5 g of hydroxylamine hydrochloride in 100mL of 95% ethanol. Store in a stoppered bottle.
5. **Ammonium hydroxide-ammonium chloride buffer:** Dissolve 67.5g of pure ammonium chloride in 570 mL of conc. Ammonia Solution and dilute to 1L. Adjust the pH at 10 using dilute NH4OH.

**Procedure**

1. Weigh 5g of air-dry soil in 250 mL conical flask.
2. Add 100 mL of saturated gypsum solution. Firmly put a rubber stopper and shake vigorously for 5 minutes.
3. Filter the contents through Whatman No. 1 filter paper. Entire quantity needs not be filtered.
4. Transfer 5mL aliquot of the clean filtrate into a 100 0r 150 mL porcelain dish.
5. Add 1 mL of the ammonium hydroxide - ammonium chloride buffer solution and 2 to 3 drops of EBT indicator.
6. Take 0.01 *N* versenate solution in a 50 mL burette and titrate the contents in the dish until the wine red colour starts changing to sky blue.
7. Run a blank using 5mL of the saturated gypsum solution in place of sample aliquot

**Calculation**

Ca or Ca + Mg (me L-1) in the aliquot = 2V

Where, V stands for volume of versenate solution used.

Since, 1 litre extract ≡ 50g soil (5g soil to 100mL),

Ca retained (or Ca requirement) in me/100g soil

= [2V for added gypsum solution -2V for filtrate] × 2 …………… (A)

Gypsum requirement of soil in tonnes per hectare up to 30cm soil depth = A × 3.852

Apply correction factor depending on purity of gypsum.

**Experiment 13: Determination of Lime Requirement of Soil**

**Principle**

In highly acidic soil having pH less than 6.0, nutrient availability is affected adversely. While availability of some elements 9like B, Mo etc.) decreases with fall in pH , that of F e , Mn , Al and many other heavy metals increases to the extent that these become toxic in certain cases. Reclaimation of acid soils, by liming, is usually practiced for keeping the soil pH in the optimum range for making nutrients available in appropriate quantities.

The amount of lime or limimg material required depends on the nature of the soil. Through liming, the exchangeable H^+ is neutralized, and the CaCO3 equivalence of the exchangeable H+ of the soil is called “lime extend to which the exchangeable H^+ is to be neutralized, as different crops demand variable soil pH for optimum growth and response. On liming , Ca^2+ and Mg^2+ replace both H^+ and Al^3+ on the colloidal surface. The liming materials [CaO or Ca(OH)2 or CaCO3]react with CO2 and water to form Ca(HCO3)2. By way of replacement of H+ and Al3+ ions, the Ca^2+ ions raise the method for determination of the lime requirement of soil(pH < 6.0)is that given by Shoemaker *et al.*(1961) which is described below:

**Instrument**

pH meter

**Reagents**

1. Extraction buffer solution: Dissolve 1.8g of nitrophenol, 2.5mL of triethanolamine, 3.0g of potassium chromate, 2.0g of calcium acetate and 53.1 of calcium chloride dihydrate in one litre of distilled water and adjust the pH to 7.5 using dilute NaOH.
2. pH buffer solutions: Solutions of pH 4.0, 7.0 and 9.2, as described under pH determination.

**Procedure**

1. Weigh 5g of air-dry soil sample in 50mL beaker.
2. Add 5Ml of distilled water and 10mL of the extractant buffer solution.
3. Stir continuously for 10 minutes or intermittently for 20 minutes with a glass rod.
4. Measure the pH of the soil-buffer suspension on a pH meter after standardizing with known pH buffer solutions.
5. Against the measured pH, find out the amount of lime required to bring the soil pH to a desired level(e.g. 6.0, 6.4 or 6.8) as given in Table 1. Make necessary correction to get the value of agricultural lime based on purity percentage.

Table 1: Lime requirement for different pH targets

Measured pH of Lime requirement in tonnes ha-1 of pure

Soil buffer suspension CaCO3 for achieving different soil pH targets

 pH 6.0 pH 6.4 pH 6.8

 67 2.43 2.92 3.40

 66 3.40 4.13 4.60

 65 4.37 5.35 6.07

 64 5.59 6.56 7.53

 63 6.56 7.78 8.99

 62 7.53 8.99 10.21

 61 8.50 10.21 11.66

 60 9.48 11.42 13.12

 59 10.69 12.64 14.58

 58 11.66 13.85 15.79

 57 12.64 15.07 17.25

 56 13.61 16.28 18.71

 55 14.58 17.50 20.17

 54 15.79 18.71 21.63

 53 16.77 19.93 22.84

 52 17.98 20.90 24.30

 51 18.95 22.11 25.76

 50 19.93 23.33 27.22

**Experiment 14: Sampling of Organic manure and fertilizer for chemical analysis**

**(i) Sample collection and preparation of Organic manure**

A composite sample should be drawn for analysis of the organic manure. Five hundred grams each from seven to eight sites from different depth of the manure pile should be collected. These should be mixed together to form a composite sample. Sub-sampling is done by spreading the manure in a circular disc-like shape and divided into four equal parts by the method known as quartering. The material in the opposite quarters is discarded and that in the remaining quarters is mixed well. This process is repeated till the sample size is reduced to approximately 500 g.

Any material such as pieces of broken glasses, stones, metal, plastics etc. present in city compost should be removed at the time of sampling. This may be clearly indicated while reporting the results. The sample thus collected should be stored in a polythene bag in a refrigerator before it is to be prepared for analysis.

In case of oilcakes, the sample is to be air dried, then powdered and passed through a 2 mm sieve before analysis (Iswaran 1980). The analysis is carried out in exactly the same way as in the case of FYM etc. Samples of press mud from sugar factories or press mud – spent wash compost can be drawn from 7 - 8 sites and for making the final sample, the procedure described above can be followed.

**Special techniques for sampling of vermicompost**

For sampling vermicompost, the living earthworms have to be separated from the compost. Vermicompost is heaped in the form of a cone under light or sunlight on a plastic sheet or on a clean surface. The earthworms move to the bottom of compost mass and can be removed. The composite sample can be drawn from the vermicompost without earthworms by the method described above for various analysis.

**Sample preparation for analysis**

Manure samples are usually partially air-dried at a temperature between 25°C to 35°C in the shade. Analyses for parameters such as pH value, ammoniacal nitrogen, nitrate nitrogen, acid extractable phosphorus, microflora and fauna should be done on moist samples and the results expressed on an oven-dry basis. For this purpose, it is important to estimate the moisture content of the sample.

* The air-dry sample is ground to pass 2 mm sieve and stored in screw capped jars.
* Analysis should be carried out as soon possible to avoid undue chemical changes subsequent to sampling.
* The microbiological analysis should be done as soon as possible using moist samples.

**(ii) Sample collection and preparation of Fertilizer**

Sample collection is a critical step in ensuring the accuracy and Validity of analytical results. While appropriate procedure is required for sampling from the bulk material at discharge ports or at manufacturer’s godowns at the production site, special care is also needed during sampling from bagged materials stored in large godowns of the producer/importers or small godowns of the trader. Various sampling procedures have been described by AOAC (2002), TFI (1982) and the FCO (1985). Details concerning solid fertilizers are presented here.

The prerequisite of good sampling is to draw as representative a sample as possible with minimum contamination and under the best possible natural condition. This requires first of all, sub division of very large stocks into identifiable smaller lots, then selection of specific representative bags on random basis to take an unbiased representative sample. Students and researchers interested in determining the nutrient content of fertilizers to be used in experiments may have to sample and analyze each bag or consignment they receive for use in research.

**General requirement of fertilizer sampling**

* The samples should not be taken at a place exposed to the rain/sun.
* The sampling instrument should be clean and dry and the sampling container should be free from any contaminations.
* The contents of each bag selected for sampling should be mixed as thoroughly as possible by suitable means to make it homogenous.
* The samples should be kept in suitable, clean, dry and air tight glass or screwed hard polyethylene bottle of about 400 g capacity or in a thick gauge polyethylene bag.

**Sampling equipment, procedure and preparation**

**Sampling equipment**

For bagged fertilizers, use a slotted single or double tube probe with solid cone tip made of stainless steel or brass having a length of about 60-65 cm with 3.2-3.8 cm outer diameter and 2.5-3.1 cm inner diameter. An example of a sampling probe is provided in Figure 1. For bulk fertilizers from conveyor belts, use stainless steel or brass sampling cup having inside dimensions of its mouth as 1.9 x 25.4 cm and length 40 cm. For bulk fertilizers in trucks, use a stainless steel or brass slotted double tube probe with solid cone tip with approximate length of 137-152 cm, outer diameter of 3.2-3.8 cm and internal diameter of 2.5-3.1. Some examples of sampling equipment have been provided in the FCO (1985).

**Sampling Procedure**

For quality control purposes, the number of bags to be chosen from a lot depends on the size. For example: if the lot consists 10 bags is chosen for sampling. The number of bags chosen increases as the size of lots increases reaching to 10 bags from a lot size of 1601-2000 bags as specified in the FCO.

The identified sampling bag should be laid horizontal and mixed thoroughly. The sampling probe should be inserted diagonally from one corner to another keeping the slit down and rotated while withdrawing. The fertilizer is emptied in a container or on a clean hard surface. Piercing at two places is required from each selected bag. All samples thus drawn are to be mixed together and reduced by the process of quartering or riffing to about 1.2 kg. Out of this, three reference samples are made and kept in clear air tight containers with identification marks.

**Sample preparation in the laboratory**

The sample received in the laboratory should be ground quickly to avoid any absorption of moisture and all material made <1 mm by repeated sieving and grinding. However, for such fertilizers whose particle size is already less than 1mm, only thorough mixing is needed to make a homogenous mass. For fertilizers mixtures, the sample should be ground in a porcelain pestle and mortar to pass through 0.425 mm IS sieve to make it more homogenous. The prepared sample should be kept in an air tight glass bottle for further analysis.

**Experiment 15: Physical properties of organic manure and fertilizers**

**Organic Manure**

**Physical and physico-chemical parameters**

* Temperature and heat out put: The maturity of a manure / compost can be judged by recording the temperature of the finished compost. It should also be free of flies, pathogens and weed seeds.
* Colour: Mature compost should be dark brown to black in colour irrespective of raw material used. Aerobic composting is characterized by progressive darkening of colour. Decomposition of biomass under anaerobic conditions terms the compost to pale green which shows minor changes with progressive composting.
* Odour: Mature compost smells like forest soil (earthy smell). This is caused by two gases (geosmin and 2-methyl lisoborneol) which are produced by fungi and actinomycetes. However, colour and odour are too subjective to provide accurate estimate of maturity. Any foul smell is not a good sign because it indicates that the composting process is not complete.
* Structure: The material should be crumbly, moderately loose, neither compact nor lumpy.
* Moisture status: The approximate moisture status of compost can be judged by inserting an iron rod at different depth in heaps or windrows. The rod would be quite moist if moisture is more than required. It can also be judged by visual observation. If it is pressed by hand, no water should drip from the sample.
* pH: A to slightly acidic reaction is most desirable although slight alkalinity is acceptable. Thus the pH of a good quality compost should be between 6.5 and 7.5. Nitrogen fixing and phosphate solubilizing bacteria can thrive well and multiply in this pH range.

Some criteria for determining the quality of compost are listed in **Table 1.**

**Table 1: Some standards for assessing the quality of compost**

|  |  |  |
| --- | --- | --- |
| **Criteria**  | **Good quality**  | **Poor quality**  |
| Colour  | Brown to black  | Varies |
| Odour (smell) | Earthy or humus like odour  | Foul colour |
| pH  | 6.5 to 7.5 | below 6 or above 8 |
| C:N Ratio  | 10:1 - 20:1 | >20:1 |
| Moisture  | About 20% | >30% |
| Temperature  | 30 to 40°C | >45°C |
| Humus | 6 - 8% | <4% |
| Nitrogen  | >1.25% | <0.1% |
| Plant Growth  | Good  | Poor inhibition  |

**Experiment 16: Determination of Total nitrogen in urea**

Various forms of N normally required to be determined in different fertilizers are (1) Total N (i) Ammoniacal N (iii) Ammoniacal and Nitrate N (iv) Urea (amide N and (v) Water insoluble N.

**Determination of total nitrogen**

Total N includes all forms of inorganic N like NH4-N, NO3-N, N, Urea-N and also the organic N compounds like proteins, amino acids and other derivatives. Depending upon the form of N present in a particular sample, specific method is to be adopted for getting the total nitrogen value. While the organic N material can be converted into simple inorganic ammoniacal salts by digestion with sulphuric acid, some chemical pre-treatment is required for reducing nitrates into ammoniacal form so that at the end of digestion, all organic and inorganic salts are converted exclusively into the ammonium sulphate form.

Kjeldhal digestion method for determining total N in nitrate-free samples is described below. It is applicable to all NO3-free straight fertilisers, ammonium phosphate and urea-based NP/NPK fertilisers & fertiliser mixtures which are nitrate free (test for the detection of nitrates in a fertiliser has been described which should help to decide whether this method should be used or not).

**Principle**

Organic nitrogenous materials when digested with H₂SO4 are oxidised to CO₂ and H2O and their inorganic N is released. During digestion part of H₂SO4is reduced to SO2 which in turn reduces nitrogenous materials to ammonia (NH3). Ammonia combines with H₂SO4 and forms (NH4)2SO4 at the end of digestion. The NH4 is distilled in alkaline medium and absorbed in standard acid. The excess of unreacted acid is back titrated with standard alkali and the amount of ammonia (as N) is calculated from the volume of standard acid consumed.

As precision of this method depends upon complete conversion of organic N into NH4-N, the digestion temperature and time, solid:acid ratio and the type of catalyst used have an important bearing on the usefulness of this method. The ideal temperature for digestion is 320°-370°C. At lower temperature, the digestion may not be complete, while above 410°C, the loss of NH3 may occur. The salt: acid (weight:volume) ratio should not be less then 1:1 at the end of digestion. Among various catalysts used to hasten the digestion are CuSO4, Hg, HgO, etc., CuSO4 being cheap even though slightly less effective, is normally used.

**Reagents**

(i) Sulphuric acid - H₂SO, (93-98%); (ii) Copper sulphate CuSO4.H2O (AR grade); (iii) Potasium sulphate or anhydrous sodium sulphate - AR grade; (iv) 45% sodium hydroxide (NaOH) solution. Dissolve 450 g solid NaOH in water and dilute to 1 litre; (v) 0.1N NaOH: Prepare 0.1N NaOH by dissolving 4.0 g NaOH in water & make volume to one liter. Standarized against 0.1 N potassium hydrogen phthalate. (vi) Zinc granule – AR grade (vii) 0.1N HCl or 0.1N H2SO4. Prepare standard 0.1N solution and standardize against 0.1N sodium carbonate or borax. (viii) Methyl red indicator solution.

**Procedure**

1. Weigh 1 g sample and place in Kjeldahl flask.
2. Add 0.7 g copper sulphate, 15 g K2SO4 or anhydrous Na2SO4 and 30 mL H2SO4.
3. Place flask in inclined position and heat gently until frothing ceases. If necessary, add small amount of paraffin to reduce frothing.
4. Boil briskly until solution is clear and then continue digestion for at least 30 more minutes.
5. Remove from burner and cool, add 200 mL water and transfer to a 500 ml volumetric flask. Cool and dilute to mark.
6. Transfer a 25 ml aliquot to distilling flask and add about 300 mL water.

**Calculations**

 **1.401 (V1N1- V₂N₂) - (V3N₁ - V4N₂)**

Percent N = -------------------------------------------------------------- × df

 W

Where

V1N1 is volume and normality of acid taken for sample respectively

V2N2 is volume and normality of alkali used for sample

V3N1 is volume and normality of acid taken in blank

V4N2is volume and normality of alkali used in blank titration

Df is dilution factor

W ml of standard acid taken in receiving flask for samples.

 ml of standard NaOH used in titrating sample

Where

 V1 = ml of standard acid taken to receiving flask for samples

V2 = ml of standard NaOH used in titrating samples

V3 = mL of standard acid taken to receiving flask for blank

V4 = ml of standard NaOH used in titrating blank

N1 = Normality of standard acid

N2 = Normality of standard NaOH

W = Weight of sample taken

df = dilution factor of sample

*Hint-1000 mL of 1N HCI= 14.01 g N*

(vii) Take accurately 20-25 ml standard acid (0.1N HCI) in the receiving conical flask so that there will be an excess of at least 5 ml of 0.1N acid. Add 2-3 drops of methyl red indicator. Add enough water to cover the end of the condenser outlet tube.

(viii) Add a few Zn granules to distillation flask to prevent bumping, till the flask and add gently 30 ml of 45% NaOH so that the contents do not mix.

(ix) Immediately connect distillation flask to distillation unit and swirl to mix the content. Distill at moderately high heat till at least 150 ml of distillate has been collected. Test with red litmus paper if any NH, is still coming out.

(x) Remove receiving flask and rinse outlet tube into receiving flask with a smal amount of distilled water.

(xi) Titrate excess standard acid in distillate with 0.1N NaOH. Determine blank on reagent using same quantity of standard acid in a receiving conical flask

**Precautions**

* The material after digestion should not solidify.
* No NH3 should be lost during distillation.
* If the indicator changes colour during distillation, repeat the determination using either a smaller sample weight or a larger volume of standard acid

**Experiment 17: Determination of total nitrogen in manure**

**Principle**

Nitro compounds formed by the reduction of salicylic acid with NO3 in acid medium are reduced to corresponding amino compounds by heating the mixture with sodium thiosulphate and zinc dust. The main product of nitration is 5-nitro salicylic acid and small amounts of 3-nitro salicylic acid.

The determination of total nitrogen in organic manure sample is commonly carried out by Kjeldahl method.

**Determination of N without nitrite and nitrate**

**Reagents**

1. Concentrated sulphuric acid (93-98%).

2. Sodium thiosulphate

3. 2% boric acid solution: Dissolve 20 g of boric acid powder in warm water by stirring and make the volume to 1 liter.

4. N/56 potassium hydrogen phthalate solution: Dissolve 3.646 g potassium hydrogen phthalate in 1 litre distilled water or 0.03646 g in 100 ml. This is the primary standard.

5. N/56 NaOH solution: Dissolve 0.7143 g NaOH in 1 litre distilled water or 0.07143 g NaOH in 100 ml distilled water. Standardise it against N/56 potassium hydrogen phthalate using phenolphthalein as an indicator.

6. N/56 H₂SO4: Dissolve 1 ml H₂SO4 in 1.5 litre of distilled water and then take 400 ml in 2 litre of distilled water. Standardize it against N/56 NaOH solution.

7. Mixed indicator: Dissolve 0.5 g bromocresol green and 0.1 g methyl red in 100 ml ethyl alcohol. Add 20 ml of this mixed indicator to 1 litre of 2% boric acid solution and adjust the pH to 4.5.

8. Catalyst mixture: 10 g K₂SO4 and 1 g CuSO4.

**Procedure**

1. Weigh 1 g manure sample and transfer it to an 800 ml Kjeldahl flask.
2. Add 25 ml sulphuric acid, 5g catalyst and 5 g sodium thiosulphate and digest at 400°C for 4 hours.

**Determination of N including nitrite and nitrate**

If nitrate and nitrate nitrogen is to be included in the estimation the following salicylic acid thiosulphate modification in the digestion procedure is required

**Reagents**

1. Sulphuric acid-salicylic acid mixture: 1 g of salicylic acid is added to 30 ml of concentrated H,SO;

2. Sodium thiosulphate.

3. Sulphate mixture: 20 parts of K2SO4 + 1 part of catalyst mixture (20 parts CuSO4 and 1 part selenium powder).

4. 40% NaOH

5. 4% boric acid.

**Procedure**

1. 1 g sample of the organic manure is transferred to a 100 ml Kjeldahl flask.
2. To the sample, 20 ml sulphuric acid-salicylic acid mixture is added and swirled gently so as to bring the dry manure sample in contact with the acid. It is allowed to stand overnight. This treatment binds the nitrate nitrogen in organic combination as follows:

(i) 2KNO3 + H2SO4 = K2SO4 + 2HNO3

(ii) HNO3 + HO-C6H4.COOH = HO-C6H3NO2.COOH + H2O

 (Nitro-salicylic acid)

After reduction, next day, 5 g of sodium thiosulphate is added and heated gently for about 5 minutes. Precaution is taken to avoid frothing. This reduces the nitrate group to form amino-salicylic acid as shown below:

(i) Na2S2O3 + H2SO4 = Na2SO4 + H2SO3 + S

(ii) 3H2SO3 + HO-C6H3NO2.COOH + H2O = 3H2SO4 + HO-C6H3NH2.COOH

 (Amino-salicylic acid)

The contents are then cooled, 10 g sulphate mixture is added to the flask and digested on the Kjeldahl apparatus at full heat. The digestion is continued for 1 hour after the solution is cleared. Bumping during digestion can be avoided by the addition of glass beads. All forms of nitrogen are converted to ammonium sulphate which when distilled with 40 % sodium hydroxide solution gives ammonia which is absorbed in standard boric acid.

(NH4)2SO4 + 2NaOH = 2NH3 + H2O + Na2SO4

**Distillation**

After digestion, the digest is cooled and diluted with distilled water to make up the volume to 100 ml.

1. 10 ml of the digest is transferred to a vacuum jacket of micro-Kjeldahl distillation.
2. In a conical flask, 10 ml of 4% boric acid containing bromocresol green and methyl red indicator to which the condenser outlet of the flask is dipped.
3. The aliquot is added and the funnel of the apparatus is washed with 2-3 ml of deionised water and 10 ml of 40 % NaOH solution is added
4. 5 ml aliquot is distilled to the flask containing 10 mL boric acid.
5. After completion of distillation, the boric acid is titrated against N/200 H2SO4until purple colour starts appearing. The decrease in strength gives the measure of nitrogen content in organic manure.
6. A blank determination of nitrogen contained in all reagents used should be carried out simultaneously to the same end point as that of the sample.

**Calculation**

The nitrogen content in the manure sample is calculated as follows:

(i) Weight of the sample = 1.0 g

(ii) Digestion volume = 100 mL

(iii) Aliquot taken = 5 mL

(iv) 1 ml of N/200 H2SO4 = 0.00007 g N

(v) Titration value (TV) = sample TV - blank TV

Example TV = 8.1 ml

 8.1 x (iv) × (ii) × 100 8.1 x 0.00007 × 100 x 100

N% = ------------------------------------ = ------------------------------------------- = 1.134%

 (i) × (iii) 1× 5

**Experiment 18: Determination of Ammoniacal-N and Nitrate-N**

The methods recommended for the determination of ammonium + nitrate (NH4-N + NO3-N) are the (i) Devarda alloy method and (ii) Ferrous sulphate zinc soda ash method.

 Zn + 2NaOH Na2ZnO2(Sodium zincate) + 2H+

Al+ NaOH Na2AlO2 (Sodium aluminate) + 2H+

NO3 + 9H+ NH3 + 3H2O

In salts containing only inorganic NH4-N and NO3-N, the nitrates are required to be reduced to ammoniacal salts before they can be distilled off with alkali. The nitrates can be reduced by zinc and ferrous sulphate in alkali medium or by zinc and Al in alkaline medium. The Devarda alloy method is described below. This method can be used for the determination of NH4-N and NO3-N, where all N is present only in form of mixtures of NO3 and NH4.

Note: It is not applicable if the samples contain urea, calcium cynamide or organic matter.

**Principle**

Devarda alloy (50% Cu, 45% Al, 5% Zn) reduces NO3 into NH4 in an alkaline condition. The NH3 formed is then distilled from alkaline solution and absorbed in a standard acid. In the alloy, Zn and Al are the reducing agents while Cu increases bitterness of the alloy and prevent bumping during distillation.

**Reagents**

1. Standard 0.1N NaOH

2. Standard 0.1N HCl or H2SO4

3. NaOH-45% solution

4. Methyl red indicator solution

5. Devarda alloy

**Procedure**

* Dissolve 1g of prepared sample in water filter, if required and make the volume to 250 ml.
* Transfer 25ml aliquot to a distilling flask, add 300ml water, 3g Devarda alloy and 5ml 45% NaOH solution pouring the latter down the side of flask so that it does not mix at once with contents.
* Immediately connect with distillation unit immersing the outlet tube of condenser in 20-25ml standard acid containing 2-3 drops metyl red. After 15 minutes, mix the contents of distilling flask by rotation.
* Heat slowly at first and then at a rate to yield 250 mL distillate in an hour. Collect distillate and titrate with standard alkali. Carry out the blank.

**Calculations**

 1.401 (V1N1- V₂N₂) - (V3N₁ - V4N₂)

NH4-N + NO3-N = -------------------------------------------------------------- × df

 W

Where V1N1 is volume and normality of acid taken for sample respectively

 V2N2 is volume and normality of alkali used for sample

 V3N1 is volume and normality of acid taken in blank

 V4N2 is volume and normality of alkali used in blank titration

df is dilution factor

 W is weight of sample taken for analysis

**Precautions**

Copper present in Devarda alloy itself works as an anti-bumping agent. However, 1-3 mL of tributyl citrate or 1 g of paraffin can be used as an anti- foaming agent, if required.

**Experiment 19: Determination of water soluble P2O5 in SSP**

**Principle**

By repeatedly leaching the fertiliser sample with water through a filter paper, the water soluble phosphate is obtained in the filtrate. The soluble orthophosphate is precipitated by quimociac reagent as quinolinium phospho molybdate (C9H7N)3H3PO4.12MoO3) which is dried at 250°C and weighed. The method is applicable to all fertilisers including fertiliser mixtures.

**Reagents**

**1.Quimociac reagent**: Dissolve 70 g of sodium molybdate dehydrate in 150 mL of water. Dissolve 60 g citric acid in mixture of 85 mL HNO3 and 150 mL water and cool. Gradually add sodium molydate solution to citric acid-nitric acid mixture with stirring. Dissolve 5 mL synthetic quinoline in mixture of 35 mL HNO3 and 100 mL water.

 Gradually add this solution to molybdate citric nitric acid solution, mix and let it stand for 24 hours and filter. Add 280 mL acetone, dilute to one litre with water and mix well. Store in a polyethylene bottle.

**2.Nitric acid AR grade (Phosphorus free).**

**Preparation of sample solution**

Place 1 g prepared sample on 9-cm filter paper No.1 or 5 in a funnel in a 250 ml volumetric flask. Using a fine stream of water directed in a circular path around the entire periphery of the filter paper, wash the sample with 10-15 ml portions until 240-250 mL filtrate has been collected within one hour. Use suction if washing would not otherwise be complete within one hour. Ensure that the water and sample are thoroughly mixed with each washing and allow each portion of water to pass through the filter before adding the next portion. If the filtrate is turbid, add 1-2 mL of HNO3 and dilute to 250 ml and mix.

**Note**: The residue lying on the filter paper is used for determination of ammonium citrate insoluble phosphorus.

**Procedure for P₂O5 estimation**

1. Pipette an aliquot containing not more than 25 mg P₂O5 (usually 25 mL) into a 500 mL beaker. Dilute if necessary to 500 mL.
2. Add 10 mL HNO3 (1+1) and boil gently for 10 minutes to hydrolyze non orthophosphates, Cool and dilute to 100 mL with water.
3. Add 50 mL quimociac reagent, shake well, cover with watch glass, place on hot plate and boil for 1 minute. **(Caution: to not use open flame).**
4. Cool to room temperature, swirl carefully 3-5 times during cooling.
5. Allow the precipitate to settle and filter through sintered glass crucible No.4. After complete transfer, wash the precipitate 5 times using 25 mL of water for each wash. Use vacuum pump. Suck dry between washings.
6. Place the crucible in a drying oven and dry at 250 for 30 minutes. Cool in a dessicator and weigh as (C9H7N)3.H3PO4.12 MoO3.
7. Carry out reagent blank and subtract the weight of reagent blank precipitate.

**Calculation**

 3.207 × A

 ------------------------ × df

 W

Where, A = Weight of precipitating (after subtraction of reagent blank precipitate)

 W = Weight of sample in g

 Df = Dilution factor (in present case 250/25 = 10)

**Experiment 20: Determination of Potassium in MOP/SOP**

Potassium (K) in all fertilizers is found mostly in water soluble form.Methods generally used for determination of K in fertilizers are

1. Volumetric STPB method
2. Gravimetric STPB method
3. Gravimetric Perchloric acid method
4. Gravimetric chloroplatinate method
5. Gravimetric cobaltinitrite method
6. Volumetric cobaltinitrite method
7. Frame photometric method

Potassium can be precipitated with Sodium Tetra Phenyl Boron (STBP) as potassium tetraphenyl borate and can be wait gravimetrically. In the volumetric method, the excess of unreacted STPB can be back titrated with zephiron chloride to estimate the quantity of STBP used for the precipitation of potassium. However, this method is susceptibleto inference for sulphates, potassium, Fe2+ and Mn2+ which have to be removed before precipitation. This is a very cumbersome method.

Potassium can also be precipitated with the help of cobalt nitrate rate and NaNO2as potassium cobaltinitrite, which can be determined either gravimetrically or volumetrically. Flame photometric method is also quite effective. However adequate precaution has to be taken on its dilution due to high concentration of potassium in the fertilizers and also use of necessary correction for its sodium content.

The STPB new metric method, which is often used by Laboratories due to its simplicity and accuracy has been described below.

**Principle**

Potassium from the fertilizer sample is first extracted with ammonium oxalate. The K in solution is precipitated with an excess of STPB as potassium tetrephenylboron. The excess of STPB is back titrated with Benzalkonium chloride (Trade Name- Zephiranchloride) or quaternary ammonium salt using Claayton yellow as indicator using clayton yellow as indicator.

Na [ B(C6H5)4] + K[B(C6H5)4] + Na+

The NH4 interferes with precipitation; hence it is first complex with formaldehyde under slightly alkaline condition before precipitation of K. The formaldehyde does not complex quaternary ammonium compounds and thus not interferes with the titration. The chlorides and sulphate d not interfere in titration.

**Reagents**

1. NaOH (20%): Dissolve 20 g and in 100 mL distilled water.
2. Formaldehyde solution 37%
3. Sodium Tetraphenyl Boron (STBP) solution (Approximately 1.2%): Dissolve 12 g sodium tetraphenyl boron in approximately 800 mL water. Add 20-25 g Al( OH)3, stir for five minutes and filter through Whatman No. 42 paper or equivalent into a 1 liter volumetric flask. Rinse the beaker sparingly with water and add to filtrate. Collect entire filtrate, add 2 mL 20% NaOH solution, dilute to volume with water and mix. Let it stand for 48 hours and standardize. Adjust so that 1 mL = 1% K2O. Store at room temperature.
4. Benzalkonium chloride (BAC) or Quaternary ammonium chloride solution (approximately 0.625%). Dilute 50 mL of 12.8% BAC to 1 liter with water, mix and standardise. Cetyl trimethyl ammonium bromide may be substituted for BAC. If other concentration is used, adjust volume accordingly.
5. Clayton yellow, 0.04 %: Dissolve 40 mg in 100 mL water.
6. Ammonium oxalate solution (NH4)₂ C₂O4%.

**Standardisation of solutions**

1. Benzalkonium chloride (BAC): Take 1.0 ml STPB solution in 250 ml Erlenmeyer flask, add 20-25 ml water, 1 ml 20% NaOH, 2.5 ml HCHO, 1.5 ml of 4% ammonium oxalate and 6-8 drops of Clayton yellow indicator. Titrate to pink end point with BAC solution so that 2.0 ml = 1.0 ml STPB solution.

2. Sodium tetraphenyl boron solution: Dissolve 2.5 g of KH₂PO4 in water in 250 ml volumetric flask, add 50 ml 4% ammonium oxalate solution, dilute to volume with water and mix. Transfer 15 ml aliquot (51.92 mg K₂O or 43.10 mg K) in 100 ml volumetric flask, add 2 ml of 20% NaOH, 5 ml HCHO, and 43 ml STPB reagent. Dilute to volume with water and mix thoroughly. Let it stand for 5-10 minutes and pass through dry filter No. 42. Transfer 50 ml aliquot of filtrate in the 250 ml Erlenmeyer flask, add 6-8 drops of indicator (Clayton yellow) & titrate excess STPB with BAC solution to pink end point.

 34.61

F = ---------------------------------------= % K₂O/ml STPB reagent

 (43.0--ml Benzalkonium)

*Note: This factor applies to all fertilisers if 2.5 g sample is diluted to 250 ml and 15 ml aliquot is taken for analysis. If results are to be expressed as K rather than K₂O, substitute 28.73 for 34.61 in calculating the value of F.*

**Procedure**

**Preparation of sample solution**

1. Straight potassium fertilisers (MOP, SOP, Potassium magnesium sulphate and Kainite). Dissolve 2.5 g prepared sample and dilute to 250 ml without adding NH4OH and (NH4)₂C₂O4. When interfering substances such as NH3, Ca, Al, etc. are present, these have to be removed before precipitation. To do this, follow the procedure given below for NPK complexes and mixtures.

2. NPK complexes and NPK fertiliser mixtures

* Place 2.5 g of prepared sample in 250 ml volumetric flask.
* Add 125 ml water and 50 ml of 4% (NH),C,O, solution. Add 1 ml diglycol stearate, to prevent foaming, if needed.
* Boil for 30 minutes, add slight excess of NH OH and after cooling dilute to 250 ml, mix and pass through dry filter No. 12 or equivalent.

**Analysis:**

* Transfer 15 ml aliquot of sample solution prepared in (a) or (b) above to 100 ml volumetric flask and add 2 ml 20% NaOH and 5 ml HCHO.
* Add 1 ml standard STPB solution for each 1% K2O expected in sample plus additional 8 ml excess to ensure complete precipitation.
* Dilute to volume with water, mix thoroughly, let it stand for 5-10 minutes and pass it through dry filter paper Whatman No.12 or equivalent. Transfer 50 ml filtrate to 250 ml Erlenmeyer flask, add 6-8 drops of indicator (Clayton yellow) and titrate excess STPB with standard Benzalkonium solution to pink end point.

**Calculation:**

% K₂O = (ml STPB – Benzalkonium used) x F

where F = % K₂O/ml of STPB reagent

*Example*

a) Calculation of factor

Suppose 43 ml of STPB was taken for determination of factor and 9.20 ml of Benzalkonium has been used in back titration. Then,

34.61 34.61

F = -----------------------= ------------------ =1.024

(43.00 – 9.20) 33.80

b) Calculation of K₂O in sample

Suppose 2.5 g sample was dilute to 250 ml and 15 ml aliquot was taken for analysis. 68.80 ml STPB was taken for analysis and 9.60 of Benzalkonium was used in back titration, then

Total K₂O% = (68.80 – 9.60) x 1.024

= 59.20 x 1.024

= 60.6208 % K₂O

**Suggestions:**

• Do not premix the NaOH and formaldehyde solution as such mixtures are not stable and lose their power to complex NH4+.

• Acetone is a good solvent for the tetraphenyl boron precipitates. Therefore, its use also helps in cleaning glassware.

• While calculation the factor as % K2O/ml of STPB, the figure of 34.61 is the actual percentage of K2O present in standard KH2PO4.

**Experiment 21: Determination of Ca and S in SSP, lime and gypsum**

**Principle**

Both calcium and magnesium can be titrated directly with EDTA using Erichrome Black (T) as indicator. However certain metals like Co, Ni, Cu, Zn d Mn cause interference which has to be either removed or masked with the use of hydroxyl amine hydrochloride or cyanide. It is also envisaged that some amount of Mg will be present in the solution, otherwise a small amount of MgCl2is to be added before EDTA titration. After the combined determination of Ca+ Mg, the Ca of the sample can be titrated with EDTA with the use of selective indicator Calcein and the Mg can then be calculated by difference. Alternatively, both Ca and Mg can be determined by AAS method.

**Reagents**

1. **Buffer solution (pH 10.0):** Dissolve 67.5 g ammonium chloride in 200 ml of distilled water, add 570 ml ammonia solution and dilute to 1 litre.

2. **Potassium hydroxide-potassium cyanide solution:** Dissolve 280 g potassium hydroxide and 66 g potassium cyanide in 1 litre of distilled water.

3. **Potassium cyanide solution (2%):** Dissolve 2 g potassium cyanide in 100 mlof distilled water.

4. **Eriochrome black T indicator solution:** Dissolve 0.2 g of indicator in 50 ml of methyl alcohol containing 2 g of hydroxyamine hydrochloride. Calcium standard solution (1 mg/ml).

5. **Calcium standard solution:** Dissolve 2.4973 g calcium carbonate (primary standard grade, previously dried for 2 hours at 285°C) in HCI (1+10). Dilute to 1 litre with double distilled water.

6. **Calcein indicator mixture:** Grind together 1 g calcein indicator with 10 g charcoal and 100 g potassium chloride.

7. **Disodium dihydrogen ethylene diamine tetra acetic acid standard solution (0.4%):** Dissolve 4 g Na2H2 EDTA in 1 litre of distilled water.

8. **Triethanolamine (1+1)**

9. **Potassium ferrocyanide solution (4%):** Dissolve 4 g potassium ferrocyanide 100 ml of distilled water.

**Standardisation of the calcium solution**

* Pipette 10 ml calcium standard solution into a 250 ml Erlenmeyer flask.Add 100 ml of distilled water, 10 ml KOH-KCN solution, 2 drops of triethanolamine solution, 5 drops of potassium ferrocyanide solution and 15± 1 mg of calcein indicator.
* Immediately place the flask on a magnetic stirrer in front of day light fluorescent light and white background. While stirring, titrate with EDTA solution to disappearance of all fluorescent green and until solution remains pink. Titrate more than 3 aliquots. From average, calculate calcium titre value.

 Volume of calcium standard solution (mL)

Calcium titre (mg/mL) = -----------------------------------------------------------------------

Volume of EDTA solution used

* From calcium titre, calculate magnesium titre value as follows:

Magnesium titre = calcium titre × 0.6064

**Estimation of Ca and Mg in sample solution**

Prepare the sample solution by weighing 1 g fertiliser sample into 250 m volumetric flask. Add 200 ml distilled water and boil for 30 minutes. Cool and dilute to volume with water and mix.

**Titration for Ca + Mg**

* Pipette 25 ml of aliquot in a 250 ml Erlenmeyer flask.
* Dilute with 100 ml of distilled water. Add 5 ml of buffer solution (pH 10), 2 mL potassium cyanide solution, 2 drops of triethanolamine solution, 5 drops of potassium ferrocyanide solution 8 drops of Eriochrome black T indicator solution.
* Titrate immediately with EDTA solution, stirring and lighting as standardization. Colour changes are wine red, purple, dark blue to clear blue to endpoint. It becomes green if over-titrated. Note the volume of EDTA used in ml (V₁).

**Titration for Ca**

* Pipette 25 ml of aliquot in an 250 ml Erlenmeyer flask.
* Dilute with 100 ml of water. Add 10 ml KOH-KCN solution, 2 drops of triethanolamine solution, 5 drops of potassium ferrocyanide solution and ± 1 mg of calcein indicator.
* Titrate immediately with EDTA solution as in standardization. Note the volume of EDTA used in ml (V₂).

(V1 – V2) ×Mg titre × 100

Mg (%) = -------------------------------------

 mg sample in aliquot

 V2 × Ca titre × 100

Ca (%) = ---------------------------------------

 mg sample in aliquot

**Experiment 22: Determination of Zn in ZnSO4 fertilizer**

The atomic absorption spectrophotometric (AAS) method is used for the analysis Zn element in Zn fertilizer. The AAS method is suitable and easy for the determination of Zn.

**Reagents**

1. Concentrated HCI

2. Demineralised double distilled water prepared from distillation of demineralised water in glass distillation set.

3. Acidified water of pH 2.5 ± 0.5: Dissolve 10 ml of 10% sulphuric acid in 10 litres of double distilled water and adjust the pH to 2.5 with the help of a pH meter by using drops of H₂SO, or NaOH.

**Preparation of standard solutions**

Prepare standard solution of 1000 ppm (µg/ml) of a given element as indicated below. Further dilute it to 0.2 to 2.0 ppm which is the working range of standard curve.

Zinc standard: Dissolve 1.0 g pure zinc metal in 30 ml of 6N HCI, heat to dissolve completely and dilute to 1000 ml. This gives 1000 ppm Zn solution. Further dilute to the range of 0.2-2.0 ppm Zn with acidified water.

**Preparation of fertiliser sample solution**

Dissolve 0.25 g of ZnSO4.7H2O in water to prepare approximately 1000 ppm solution. Dilute further to fit in the suitable standard curve range of 0.2 to 2.0 ppm with acidified water.

**Flaming of the solution**

Flame the standard and the sample solution in AAS at corresponding wavelength and hollow cathode lamp and draw the graph of concentration vs absorbance. Read the concentration of sample solution from standard curve (x ppm).

**Calculation**

 X ppm × dilution factor × 10-4

Zn (%) = ---------------------------------------------------------

 Weight of sample (g)